## **RESULTS**

## 1- Collection and isolation of pressure sores bacteria.

Bacterial organisms isolated from 35 patients (21 male and 14 female) with pressure sores occur in different parts of the body, as illustrated in photos No. (1), by using sterile swaps on nutrient's agar and blood agar medium.

The bacteria were isolated from different age, from 45-75 years of male patient and from 35-65 years of female patient and the percent of bacterial isolates were recorded in table (1&2) and figure (1). These results illustrated that the highest percentage obtained was 33.4 % of positive samples found in male ages from 71-75 years and the highest percentage of female sample reaches to 28.6 % ages from 56-60 years.

Table (1): Bacterial organisms isolated from different ages of bed sores males.

Age of males (year)	No. of patients	Patient ( % )
45-50	1	4.7
51-55	3	14.3
56-60	3	14.3
61-65	1	4.7
66-70	6	28.6
71-75	7	33.4
Total	21	100.0

Table (2): Bacterial organisms isolated from different ages of bed sores females.

Age of females (year)	No. of patients	Patient ( % )
35-40	2	14.2
41-45	1	7.2
46-50	1	7.2
51-55	3	21.4
56-60	4	28.6
61-65	3	21.4
Total	14	100.0

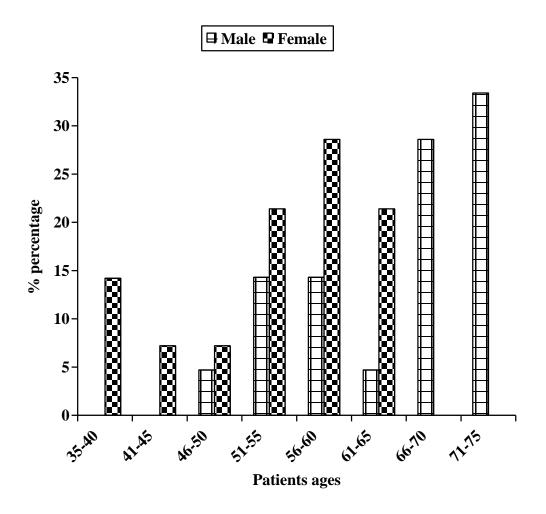


Fig. (1): Bed sores percentages in males and females with different ages.



Photos No. (1): Patients with bedsores on different parts of the body.

#### 2- Identification of bacterial isolates

The clinical isolates were subjected to two patterns of identification according to the Bergey's Manual of Determinative Bacteriology (**Holt** *et al*, **1994**), as illustrated in table (3) and photos No. (2).

First the staining reactions and the second is culture characteristics of isolates on simple, enriched and selective media as well as biochemical reactions.

According to Gram stain methods, 23 isolates were Gram-negative bacilli, while 12 isolates were Gram-positive cocci.

#### I- Gram-negative bacilli (23 isolates)

#### 1- The first type of Gram-negative bacilli isolates: includes 3 isolates

Colonial morphology: Circular, convex, smooths colonies with distinct edges.

Cultural properties: Facultative anaerobic, produce rose-pink to red colonies on MacConkey agar media, so it is lactose fermentor. All isolates showed yellow slant and yellow butt (acidic for each) with gas bubbles in triple sugar iron agar (TSI) test.

**Gram staining and microscopic examination:** Gram-negative bacilli without special arrangement, non-capsulated and non spore-forming.

**Biochemical characteristics:** Produce catalase enzyme, potassium hydroxide test positive, motile, produce indole, and give positive methyl-red reaction and negative Voges-Proskauer reaction, urease and citrate are negative; H<sub>2</sub>S and oxidase test are negative.

**Carbohydrates fermentation:** Glucose is positive, sucrose is positive, maltose is positive, lactose is positive and mannitol is positive.

According to the above characteristics the 3 isolates of the first type of Gram-negative bacilli were identified as strains belonging to *Escherichia coli*.

#### 2- The second type of Gram-negative bacilli isolates: include 2 isolates

Colonial morphology: Round, slightly large, undulate, unbenate and produce mucoid colonies on media rich with sugar like MacConkey agar media.

Cultural properties: Facultative anaerobic, produce rose-pink to red mucoid colonies on MacConkey agar media, so it is lactose fermentor. All isolates showed yellow slant and yellow butt (acidic for each) with gas bubbles in triple sugar iron agar (TSI) test.

**Gram stain and microscopic examination:** Gram negative bacilli with rounded ends, non spore-forming, encapsulated.

**Biochemical characteristics:** Potassium hydroxide test positive, produce catalase enzyme, non-motile, indole test negative, give negative methyl-red reaction and positive Voges-Proskauer reaction, urease and citrate are positive, H<sub>2</sub>S and oxidase test are negative.

**Carbohydrates fermentation:** Glucose is positive, sucrose is positive, lactose is positive, maltose is positive and mannitol is positive.

According to the above characteristics the 2 isolates of the second type of Gram-negative bacilli were identified as strains belonging to *Klebsiella pneumoniae*.

#### 3- The third type of Gram-negative bacilli isolates: include 10 isolates

**Colonial morphology:** Round, convex, small, with entire margin, tan and mucoid colonies.

**Cultural properties:** Aerobic and facultative anaerobic produce swarming growth on nutrient agar and blood agar, colorless colonies without swarming on MacConkey agar, so it is non-lactose fermentor. All isolates showed yellow slant and yellow-black butt (acidic for each) in triple sugar iron agar (TSI) test.

**Gram staining and microscopic examination:** Gram-negative bacilli, non-capsulated, pleomorphic and non spore-forming.

**Biochemical characteristics:** Potassium hydroxide test positive, produce catalase enzyme, motile, indole test positive, give positive methyl-red reaction and negative Voges-Proskauer reaction, urease positive, citrate negative, H<sub>2</sub>S test are positive and oxidase test are negative.

Carbohydrates fermentation: Glucose is positive, lactose is negative, some strains are positive and some strains are negative for maltose, sucrose and mannitol.

According to the above characteristic the 10 isolates of the third type of Gram-negative bacilli were identified as strains belonging to *Proteus vulgaris*.

### 4- The fourth type of Gram-negative bacilli isolates: include 8 isolates

**Colonial morphology:** Flat, small and rough colonies, grayish-green to bluish colonies with mucoid texture and irregular margins.

Cultural properties: Strict aerobic, produce water soluble (diffusible) green, blue, or other color pigments which called diffusible pigments. Almost all of these isolates produced green diffusible pigment on nutrient agar, MacConkey agar, it is non-lactose fermentor. All isolates showed red slant and red butt (alkaline for each) in triple sugar iron agar (TSI) test.

**Gram staining and microscopic examination:** Gram-negative bacilli, straight rods, non-capsulated and non spore-forming.

**Biochemical characteristics:** Potassium hydroxide test positive, produce catalase enzyme, motile, indole test negative, give negative methyl-red reaction and negative Voges-Proskauer reaction, urease are negative and citrate are positive, H<sub>2</sub>S test are negative and oxidase test are positive.

Carbohydrates fermentation: Glucose is positive incase oxidation only, lactose are negative, sucrose and mannitol are negative and maltose are positive.

According to the above characteristic the 8 isolates of the fourth type of Gram-negative bacilli were identified as strains belonging to *Pseudomonas aeruginosa*.

#### II- Gram-positive cocci (12 isolates)

## 1- The first type of Gram-positive cocci isolates: includes 11 isolates

**Colonial morphology:** Smooth, convex, circular colonies with white color.

**Cultural properties:** Facultative anaerobic, Gram staining and microscopic examination: Gram-positive cocci, arranged in clusters, non-capsulated and non spore-forming.

**Biochemical characteristics:** Potassium hydroxide test negative, produce catalase enzyme, non-motile, no growth on macConkey's media, non-coagulase, can't grow on manitol salt agar and sensitive to novobiocin.

Carbohydrates fermentation: Glucose and mannitol are positive.

According to the above characteristic the 11 isolates of the first type of Gram-positive cocci were identified as strains belonging to *Staphylococcus epidermidis*.

### 2- The second type of Gram-positive cocci isolates: includes one isolates

**Colonial morphology:** Smooth, convex, circular colonies with yellow color.

**Cultural properties:** Facultative anaerobic, Gram staining and microscopic examination: Gram-positive cocci, arranged in clusters, non-capsulated and non spore-forming.

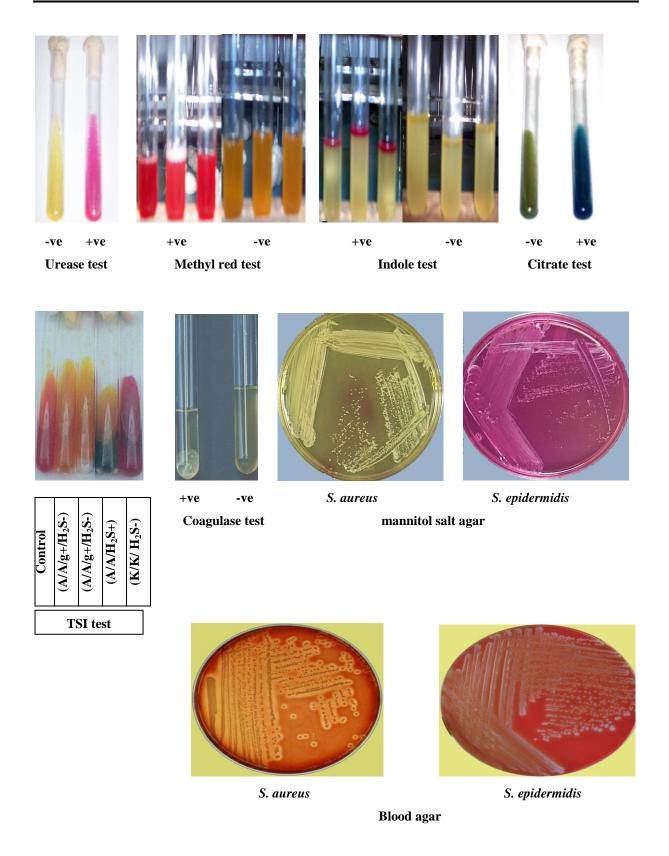
**Biochemical characteristics:** Potassium hydroxide test negative, produce catalase enzyme, non-motile, no growth on macConkey's media, coagulase positive, can grow on mannitol salt agar and also produce positive result, sensitive to novobiocin antibiotics.

Carbohydrates fermentation: Glucose and mannitol are positive.

According to the above characteristic the one isolate of the second type of Gram-positive cocci were identified as strains belonging to *Staphylococcus* aureus.

Table (3): Biochemical reaction of E. coli, Klebsiella pneumoniae, Proteus vulgaris, Pseudomonas aeruginosa, Staphylococcus aureus and Staphylococcus epidermidis

<b>Biochemical test</b>	E. coli	K. pneumoniae	P. vulgaris	P. aeruginosa	S. epidermidis	S. aureus
Gram stain	-ve	-ve	-ve	-ve	+ve	+ve
Capsule stain	-ve	+ve	-ve	-ve	-ve	-ve
KOH test	+ve	+ve	+ve	+ve	-ve	-ve
Catalse test	+ve	+ve	+ve	+ve	+ve	+ve
Oxidase test	-ve	-ve	-ve	+ve		
Lactose	+ve	+ve	-ve	-ve		
Glucose	+ve	+ve	+ve	+ve in O <sub>2</sub>	+ve	+ve
Maltose	+ve	+ve	<u>+</u> ve	-ve		
Sucrose	+ve	+ve	<u>+</u> ve	-ve		
Mannitol	+ve	+ve	<u>+</u> ve	-ve	-ve	+ve
$H_2S$	-ve	-ve	+ve	-ve		
Indole test	+ve	-ve	-ve	-ve		
Methyl red	+ve	-ve	+ve	-ve		
Voges proskauer	-ve	+ve	-ve	-ve		
Citrate utilization	-ve	+ve	+ve	+ve		
Urease	-ve	+ve	+ve	-ve		
Nitrate reduction	+ve	+ve	+ve	-ve		
Motility	Motile	Non-motile	Motile	Motile	Non-motile	Non-motile
Novobiocin susceptibility					sensitive	sensitive
Coagulase test					-ve	+ve



Photos No. (2): Biochemical reactions used in identification of bacterial isolates.

### 3- The distribution of pathogenic isolates from pressure ulcer samples

The results in table (4) and fig. (2), indicated that the number of contaminated pressure ulcer samples collected from males and females were 11 of *S. epidermidis*,10 of *Proteus vulgaris*, 8 of *Pseudomonas aeruginosa*, 3 of *E. coli*, 2 of *Klebsiella pneomoniae* and one of *S. aureus*.

So the highest percentages of distribution are found in *S. epidermidis* (31.4%) followed by *Proteus vulgaris* (28.6%), *Pseudomonase aeruginosa* (22.8%). *E. coli* (8.6%), *Klebsiella pneumoniae* (5.8%) and *S. aureus* (2.8%).

Table (4): The distribution number of pathogenic bacterial isolates from pressure ulcer collected samples.

Pathogenic bacterial isolates	No. Of bacterial isolates	Percentage of distribution,
E. coli	3	8.6
P. vulgaris	10	28.6
K. pneumoniae	2	5.8
S. epidermidis	11	31.4
P. aeruginosa	8	22.8
S. aureus	1	2.8
Total	35	100.0

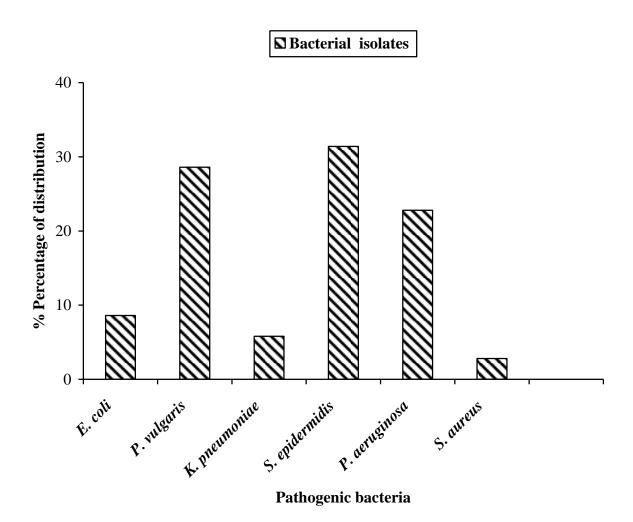


Fig. (2): The distribution number of pathogenic bacterial isolates from pressure ulcer collected samples.

# 4- Susceptibility of different pathogenic isolates to different antibiotics drugs

Different commercial antibiotics used to show their effect on pathogenic isolated bacterial organisms causing pressure sores. The antibiotics used were penicillin (P) (10 $\mu$ g), ampicillin (AM) (10 $\mu$ g), erythromycin (E) (15 $\mu$ g), norfloxacin (NOR) (10 $\mu$ g), ofloxacin (OFX) (5 $\mu$ g), amikacin (AK) (30 $\mu$ g), chloramphenicol (C) (30 $\mu$ g), cephalexin (CL) (30 $\mu$ g), as illustrated in table (5) and photos No. (3).

The antibiotics of loxacin with concentration (5  $\mu$ g/disc) showed the highest effect against all tested pathogenic bacteria followed by, norfloxacin with concentration (10  $\mu$ g/disc) showed moderate effect followed by chloramphenicol with concentration (30 $\mu$ g) and Amikacin with concentration (10 $\mu$ g).

The pathogenic bacterial isolates E. coli number 11 showed resistant against antibiotics penicillin, ampicillin, cephalexin, erythromycin, chloramphenicol, ofloxacin and norfloxacin, Proteus vulgaris number 14 showed resistant against antibiotics penicillin, ampicillin, erythromycin, chloramphenicol, ofloxacin. Amikacin and norfloxacin and Pseudomonas aeruginosa number 13 showed resistant against antibiotics penicillin, ampicillin, cephalexin, erythromycin, chloramphenicol, ofloxacin and norfloxacin, and Klebsiella pneumoniae number 25 showed resistant against antibiotics penicillin, ampicillin, cephalexin, erythromycin, chloramphenicol, ofloxacin and norfloxacin, E. coli number 11, Proteus vulgaris number 14 and Pseudomonas aeruginosa number 13 indicated the highest clinical resistance against antibiotics penicillin, ampicillin, cephalexin, erythromycin, ofloxacin, norfloxacin, amikacin and chloramphenicol.

Table (5): Inhibition zone (mm) of different pathogenic bacterial isolates by different tested antibiotics

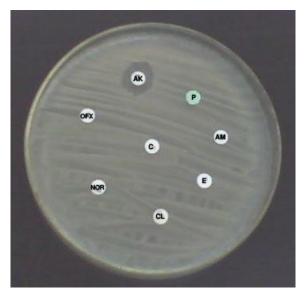
No.	Pathogenic														
110.	isolates					Dia	amete	r of inhibi	tion zo	nes (n	ım)				
	isolates	NO	R	0	FX	(	7	CL		E	Α	M	Α	K	P
		(10)			μg)	(30		(30 µg)	(15	μg)		0 μg)		μg)	(10 μg)
		IZ	ST	IZ	ST	IZ	ST	IZ ST	IZ	ST	IZ	ST	IZ	ST	IZ ST
1	S. epidermidis	22	S	25	S	32	S	ND	35	S		D	23	S	ND
2	S. epidermidis	13	I	20	S	25	S	ND	22	Ι	25	S	18	S	ND
3	P. aeruginosa	29	S	25	S	N	D	ND	N	D	N	D	22	S	ND
4	P. aeruginosa	27	S	22	S	N	D	ND	18	Ι	N	D	21	S	ND
5	S. epidermidis	20	S	20	S	33	S	ND	25	S	N	D	22	S	ND
6	P. vulgaris	14	I	15	I	9	R	ND	10	R		D	N	D	ND
7	S. epidermidis	27	S	33	S	31	S	15 R	25	S	33	S	29	S	ND
8	S. epidermidis	21	S	23	S	30	S	ND	28	S		D	21	S	ND
9	P. aeruginosa	20	S	22	S	N		ND	10	R	10	R	9	R	ND
10	S. epidermidis	25	S	30	S	25	S	13 S	20	I	15	S	19	S	ND
11	E. coli	NI			D	N		ND		D		D	16	I	ND
12	E. coli	17	S	22	S	23	S	ND	15	I		D	16	I	ND
13	P. aeruginosa	NI			D	N		ND		D		D	12	I	ND
14	P. vulgaris	NI			D	N		ND		D		D		D	ND
15	S. epidermidis	NI		18	S	23	S	ND		D	20	S	14	R	ND
16	P. vulgaris	25	S	25	S	N		ND		D		D	18	S	ND
17	P. aeruginosa	NI		9	R	18	S	ND	10	R		D	16	I	ND
18	P. vulgaris	18	S	20	S	8	R	ND	18	I		D		D	ND
19	P. aeruginosa	18	S	20	S	N		ND	12	R		D	16	I	ND
20	S. epidermidis	NI		12	R	25	S	ND		D	26	S	12	R	ND
21	K. pneumoniae	28	S	30	S	25	S	16 I	25	S		D	20	S	ND
22	P. vulgaris	NI 25		10 35	R	10	R	ND 22 S	12 19	R		D	15	I	ND
23	E. coli	35 18	S	28	S	30 N	S	22 S ND	_	I D	30	D	16 19	S	ND ND
25	P. vulgaris	NI			D S	N		ND ND	_	D		D	12	R	ND ND
26	K. pneumoniae P. vulgaris	21	S	16	S	9	R	9 R	8	R	8	R	8	R	ND ND
27	P. aeruginosa	NI NI			D	11	R	ND	_	D D	15	I	13	R	ND
28	P. aeruginosa	NI		12	R	N		ND ND		D		D	8	R	ND
29	S. epidermidis	20	S	28	S	30	S	ND ND	33	S		D	22	S	ND
30	S. epidermidis	14	I	18	S	28	S	ND ND	24	S	16	ז <u>ו</u>	20	S	ND
31	P. vulgaris	32	S	30	S	N		ND ND		D	12	R	20	S	ND
32	S. epidermidis	22	S	25	S	30	S	ND	25	S		D	23	S	ND
33	P. vulgaris	17	S		D	20	S	ND	_	D	15	I	20	S	ND
34	P. vulgaris	20	S	20	S	20	S	ND	15	I	10	R	18	S	ND
35	S. aureus	22	S	25	S	25	S	ND	25	S	20	S	20	S	ND
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Penicillin = P, Ampicillin = AM, Erythromycin = E, Norfloxacin = NOR, Ofloxacin = OFX, Amikacin = AK, Chloramphenicol = C & Cephalexin = CL.

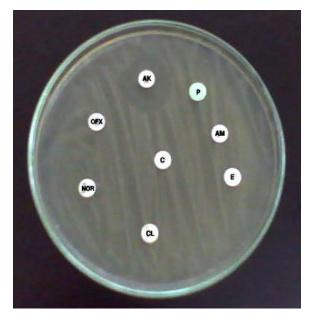
Susceptibility test = ST, Inhibition zone (mm) = IZ, Not detected (0.00) = ND, Sensitive = S, Resistance = R & Intermediate = I.



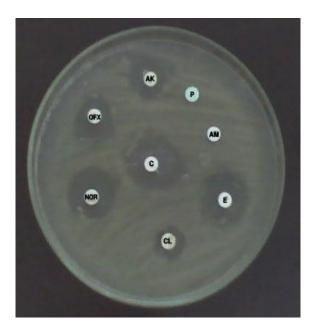
S. epidermidis number 10



E. coli number 11



Pseudomonas aeruginosa number 13



K. pneumoniae number 21

Photos No. (3): Antibiotic susceptibility of bacterial isolates by disc diffusion method.

AK = amikacin, P = penicillin, AM = ampicillin, E = erythromycin, CL = cephalexin, NOR = norfloxacin, OFX = ofloxacin & C = chloramphenicol.

#### 5- Sensitivity of pathogenic isolates to tested antibiotics.

The sensitivity tests of pathogenic bacterial isolates against different tested antibiotics were illustrated in table (6) and fig. (3). These results showed that the antibiotic ofloxacin is more effective against isolated pathogenic bacterial organisms, which the percentage of sensitive organism reach to 68.6% followed by norfloxacin 62.8%, chloramphenicol and amikacin 51.4%, erythromycin 25.7%, ampicillin 20.0%, cephalexin 5.8% and penicillin not affected on all bacterial isolates.

The antibiotic amikacin and erythromycin show intermediate effect against isolated pathogenic bacterial organisms, where the percentage reaches to 20.0% followed by ampicillin and norfloxacin 8.6%, ofloxacin and cephalexin 2.8% and chloramphenicol and penicillin 0.0%. On the other hand, the antibiotic penicillin hasn't any effect against isolated pathogenic bacterial organisms, where the percentage of resistance organisms reach to 100.0% followed by cephalexin 91.4%, ampicillin 71.4%, erythromycin 54.3%, chloramphenicol 48.6% and norfloxacin, ofloxacin and amikacin 28.6%.

Table (6): Sensitivity of pathogenic isolates to different antibiotics drugs.

Tested antibiotics		itive ates		mediate lates	Resistance isolates		
	No.	%	No.	%	No.	%	
Norfloxacin (NOR)	22	62.8	3	8.6	10	28.6	
Ofloxacin (OFX)	24	68.6	1	2.8	10	28.6	
Chloramphenicol (C)	18	51.4	0	0.0	17	48.6	
Cephalexin (CL)	2	5.8	1	2.8	32	91.4	
Erythromycin (E)	9	25.7	7	20.0	19	54.3	
Ampicillin (AM)	7	20.0	3	8.6	25	71.4	
Amikacin (AK)	18	51.4	7	20.0	10	28.6	
Penicillin (P)	0	0.0	0	0.0	35	100.0	

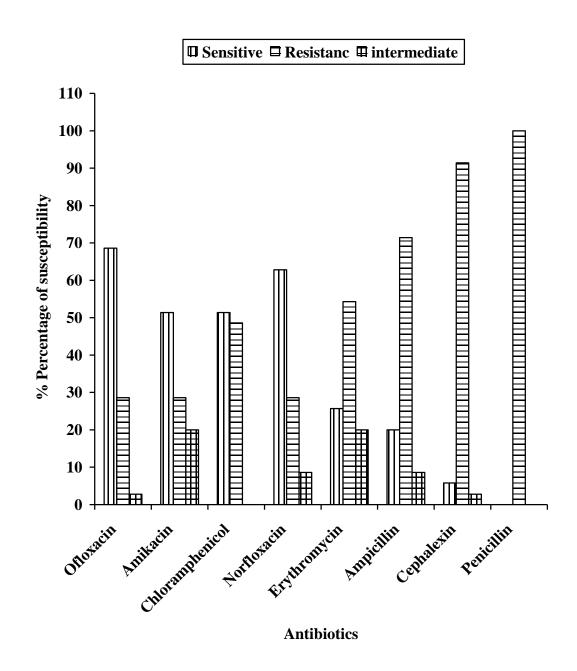


Fig. (3): The sensitivity test of pathogenic bacterial isolates to different antibiotics drugs.

# 6- Statistical analysis of sensitive, intermediate and resistant pathogenic bacterial isolates against different antibiotics.

The obtained results in table (7 a, b, c, d, e, f, g and h) showed that the sensitivity of pathogenic bacterial isolates against each tested antibiotic to illustrate the obtained results are significant or not.

The statistical analysis incase chloramphenical and erythromycin are highly significant, while incase ampicillin is significant, but incase ofloxacin, amikacin, norfloxacin and cephalexin are non significant.

These results clearly indicated that *Proteus vulgaris* showed highly resistant against most tested antibiotics drugs which recorded the percentage of resistant 30.0, 40.0, 47.2, 31.2, 28.6, 20.0, 44.4 and 36.0 against ofloxacin, amikacin, chloramphenicol, cephalexin, penicillin, norfloxacin, erythromycin and ampicillin, respectively followed by *Pseudomonas aeruginosa*, *Staphylococcus epidermidis*, *E. coli*, *Klebsiella pneumoniae* and *S. aureus* showed the lowest resistance against tested antibiotics drugs. On the other hand the antibiotic cephalexin has the lowest effect against tested clinical bacterial isolates, and penicillin not recorded any sensitivity and intermediate effect against tested clinical bacterial isolates.

Table (7): Statistic analysis of sensitive, intermediate and resistant pathogenic bacterial isolates to different antibiotics.

## a) Antibiotic ofloxacin (OFX)

<b>Bacterial isolates</b>	Sens	itive	Interm	ediate	Resi	stant	$\mathbf{X}^2$	P
Dacterial Isolates	No.	%	No.	%	No.	%	Λ	
E. coli	2	8.2	0	0.0	1	10.0		
P. vulgaris	6	25.0	1	1.0	3	30.0		0.42
K. pneumoniae	1	4.2	0	0.0	1	10.0	4.95	
P. aeruginosa	4	16.7	0	0.0	4	40.0	4.95	0.42 NS
S. epidermidis	10	41.7	0	0.0	1	10.0		110
S. aureus	1	4.2	0	0.0	0	0.0		
Total	24	100.0	1	100.0	10	100.0		

## b) Antibiotic amikacin (AK)

Bacterial isolates	Sens	itive	Intern	nediate	Res	istant	$\mathbf{X}^2$	P
Dacterial isolates	No.	%	No.	%	No.	%	Λ	Г
E. coli	0	0.0	3	42.9	0	0.0		
P. vulgaris	5	27.7	1	14.2	4	40.0		0.06
K. pneumoniae	1	5.6	0	0.0	1	10.0	10.43	
P. aeruginosa	2	11.1	3	42.9	3	30.0	10.43	NS
S. epidermidis	9	50.0	0	0.0	2	20.0		110
S. aureus	1	5.6	0	0.0	0	0.0		
Total	18	100.0	7	100.0	10	100.0		

## c) Antibiotic chloramphenicol (C)

Bacterial isolates	Sens	Sensitive		ediate	Resi	stant	$\mathbf{X}^2$	P
	No.	%	No.	%	No.	%	Λ	Г
E. coli	2	11.1	0	0.0	1	5.8		
P. vulgaris	2	11.1	0	0.0	8	47.2		
K. pneumoniae	1	5.6	0	0.0	1	5.8	20.42	0.001
P. aeruginosa	1	5.6	0	0.0	7	41.2	20.42	HS
S. epidermidis	11	61.0	0	0.0	0	0.0		
S. aureus	1	5.6	0	0.0	0	0.0		
Total	18	100.0	0	0.0	17	100.0		

# d) Antibiotic norfloxacin (NOR)

D4	Sens	itive	Intern	nediate	Resi	stant	2	
Bacterial isolates	No.	%	No.	%	No.	%	$\mathbf{X}^2$	P
E. coli	2	9.2	0	0.0	1	10.0		
P. vulgaris	7	31.8	1	33.3	2	20.0	1.54	0.9
K. pneumoniae	1	4.5	0	0.0	1	10.0		
P. aeruginosa	4	18.2	0	0.0	4	40.0	1.34	NS
S epidermidis	7	31.8	2	66.7	2	20.0		1 10
S. aureus	1	4.5	0	0.0	0	0.0		
Total	22	100.0	3	100.0	10	100.0	·	

# e) Antibiotic erythromycin (E)

Bacterial	Sens	sitive	Intern	ediate	Resi	stant	$\mathbf{X}^2$	P
isolates	No.	%	No.	%	No.	%	Λ	r
E. coli	0	0.0	2	25.0	1	5.6		0.0010
P. vulgaris	0	0.0	2	25.0	8	44.4		
K. pneumoniae	1	11.1	0	0.0	1	5.6	19.06	
P. aeruginosa	0	0.0	2	25.0	6	33.3	19.00	0.0018 HS
S. epidermidis	7	77.8	2	25.0	2	11.1		
S. aureus	1	11.1	0	0.0	0	0.0		
Total	9	100.0	8	100.0	18	100.0		

# f) Antibiotic ampicillin (AM)

Bacterial isolates	Sensitive		Intern	nediate	Resi	stant	$\mathbf{X}^2$	P
Dacterial isolates	No.	%	No.	%	No.	%	Λ	r
E. coli	1	14.3	0	0.0	2	8.0		
P. vulgaris	0	0.0	1	33.3	9	36.0		0.017
K. pneumoniae	0	0.0	0	0.0	2	8.0	12.70	
P. aeruginosa	0	0.0	1	33.3	7	28.0	13.79	0.017 S
S. epidermidis	5	71.4	1	33.3	5	20.0		b
S. aureus	1	14.3	0	0.0	0	0.0		
Total	7	100.0	3	100.0	25	100.0		

# g) Antibiotic cephalexin

Bacterial isolates	Sens	ensitive Intermediate Resistant				stant	$\mathbf{X}^2$	P
Dacterial Isolates	No.	%	No.	%	No.	%	Λ	Г
E. coli	1	50.0	0	0.0	2	6.2		0.33
P. vulgaris	0	0.0	0	0.0	10	31.2		
K. pneumoniae	0	0.0	1	100.0	1	3.2	5.75	
P. aeruginosa	0	0.0	0	0.0	8	25.0	5.75	0.33 NS
S. epidermidis	1	50.0	0	0.0	10	31.2		110
S. aureus	0	0.0	0	0.0	1	3.2		
Total	2	100.0	1	100.0	32	100.0		

# h) Antibiotic penicillin

Bacterial isolates	Sens	sitive	Interm	ediate	Resi	stant	$\mathbf{X}^2$	P
Dacterial Isolates	No.	%	No.	%	%	Λ	r	
E. coli	0	0.0	0	0.0	3	8.6		
P. vulgaris	0	0.0	0	0.0	10	28.6		
K. pneumoniae	0	0.0	0	0.0	2	5.7		
P. aeruginosa	0	0.0	0	0.0	8	22.8		
S. epidermidis	0	0.0	0	0.0	11	31.5		
S. aureus	0	0.0	0	0.0	1	2.8		
Total	0	0.0	0	0.0	35	100.0		

# 7- Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of some antibiotics.

The experimental bacteria were treated separately with different concentration of the antibiotics under test in nutrient broth medium and the MICs and the MBCs were determined.

The results in table (8, a) indicated that the maximum MBC were obtained at ofloxacin antibiotic which recorded 250 μg/ml against *P. aeruginosa* number 13 & 27 and *Proteus vulgaris* number 14 and the lowest MBC were obtained 31.25 μg/ml at *S. epidermidis* number 10 & 7 and *S. aureus* 35. MBC equal to MIC which recorded 250 μg/ml of ofloxacin against *Proteus vulgaris* number 14, 125 μg/ml of ofloxacin against *Klebsiella pneumoniae* number 25 and *E. coli* number 11, 62.5 μg/ml of ofloxacin against *Proteus vulgaris* number 22 and 31.25 μg/ml of ofloxacin against *S. epidermidis* number 7.

The results in table (8, b) indicated that the maximum MBC were obtained at norfloxacin antibiotic which recorded 250 μg/ml against *E. coli* number 11, *P. aeruginosa* number 28 & 27, *K. pneumoniae* number 25 and *Proteus vulgaris* number 22, and the lowest MBC were obtained 31.25μg/ml at *S. epidermidis* number 7 & 10. MBC equal to MIC which recorded 125 μg/ml of norfloxacin against *Proteus vulgaris* number 14 and *P. aeruginosa* number 13; and 31.25 μg/ml of norfloxacin against *S. epidermidis* number 7.

The results in table (8, c) indicated that the maximum MBC were obtained at chloramphenicol antibiotic which recorded 250 µg/ml against *E. coli* number 11, *P. aeruginosa* number 13, 27 & 28, *K. pneumoniae* number 25 and *Proteus vulgaris* number 14, and the lowest MBC were obtained 62.5 µg/ml at *S. epidermidis* number 7 & 10. MBC equal to MIC which recorded 250 µg/ml

of chloramphenicol against *P. aeruginosa* number 13, 27 & 28, *E. coli* number 11 and *Proteus vulgaris* number 14, and 62.5 μg/ml of chloramphenicol against *S. epidermidis* number 10 & 7.

The results in table (8, d) indicated that the maximum MBC were obtained at amikacin antibiotic which recorded 250 µg/ml against *P. aeruginosa* number 27, and *Proteus vulgaris* number 14, and the lowest MBC were obtained 31.25 µg/ml at *S. epidermidis* number10 and *K. pneumoniae* number 21. MBC equal to MIC which recorded 125 µg/ml of amikacin against *P. aeruginosa* number 28.

The statistical analysis for selected antibiotics (MIC & MBC) incase norfloxacin is significant, but incase ofloxacin, amikacin, and chloramphenicol are non significant.

Table (8): Minimum inhibitory concentration (MICs) ( $\mu g/ml$ ) and minimum bactericidal concentration (MBCs) of selected antibiotics.

### a) Antibiotic ofloxacin

Do otowial isolates	Serial	(MIC)	(MBC)
Bacterial isolates	No.	(µg/ml)	(µg/ml)
P. vulgaris	14	250	250
P. aeruginosa	13	125	250
E. coli	11	125	125
S. epdirmidis	10	7.813	31.25
P. aeruginosa	28	62.5	125
S. epdirmidis	7	31.25	31.25
P. vulgaris	22	62.5	62.5
S. aureus	35	7.813	31.25
K. pneumoniae	25	125	125
K. pneumoniae	21	31.25	62.5
P. aeruginosa	27	125	250
$\overline{X}$ +SD		86.6 <u>+</u> 72	122.1 <u>+</u> 89.99
t			.02
P		0.3	2 NS

## b) Antibiotic norfloxacin

Do otowial igalates	Serial	(MIC)	(MBC)
Bacterial isolates	No.	(µg/ml)	(µg/ml)
P. vulgaris	14	125	125
P. aeruginosa	13	125	125
E. coli	11	125	250
S. epdirmidis	10	15.625	31.25
P. aeruginosa	28	125	250
S. epdirmidis	7	31.25	31.25
P. vulgaris	22	125	250
S. aureus	35	15.625	62.5
K. pneumoniae	25	125	250
K. pneumoniae	21	31.25	125
P. aeruginosa	27	125	250
$\overline{X}$ +SD		88.1 <u>+</u> 51	159.1 <u>+</u> 93
t		2	.21
P		0.0	36 S

# c) Antibiotic chloramphenicol

	G . 1	(MITC)	(A (D C))
<b>Bacterial isolates</b>	Serial	(MIC)	(MBC)
Dacter far isolates	No.	(µg/ml)	$(\mu g / ml)$
P. vulgaris	14	250	250
P. aeruginosa	13	250	250
E. coli	11	250	250
S. epdirmidis	10	62.5	62.5
P. aeruginosa	28	250	250
S. epdirmidis	7	62.5	62.5
P. vulgaris	22	62.5	125
S. aureus	35	62.5	125
K. pneumoniae	25	125	250
K. pneumoniae	21	62.5	125
P. aeruginosa	27	250	250
$\overline{X}$ +SD		153.4 <u>+</u> 94	181.8 <u>+</u> 81
t		0	.75
P		0.:	5 NS

# d) Antibiotic amikacin

Postavial igalates	Serial	(MIC)	(MBC)
<b>Bacterial isolates</b>	No.	(µg/ml)	(µg /ml)
P. vulgaris	14	125	250
P. aeruginosa	13	62.5	125
E. coli	11	62.5	62.5
S. epdirmidis	10	31.25	31.25
P. aeruginosa	28	125	125
S. epdirmidis	7	31.25	62.5
P. vulgaris	22	62.5	125
S. aureus	35	62.5	62.5
K. pneumoniae	25	62.5	125
K. pneumoniae	21	31.25	31.25
P. aeruginosa	27	125	250
$\overline{X} + SD$		71 <u>+</u> 37	113.7 <u>+</u> 76.9
t			.65
P		0.1	1 NS

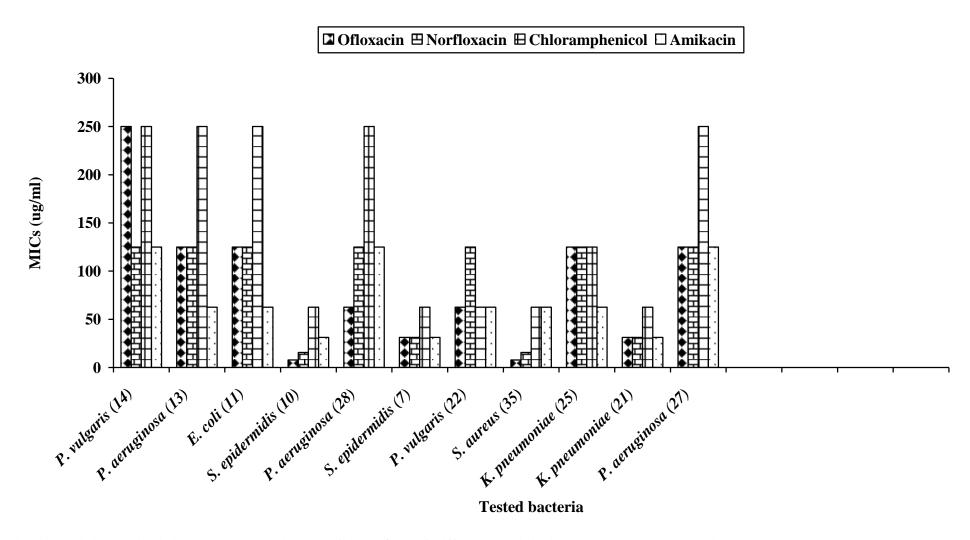


Fig. (4): Minimum inhibitory concentration (MICs) (µg/ml) of different antibiotics against tested bacteria.

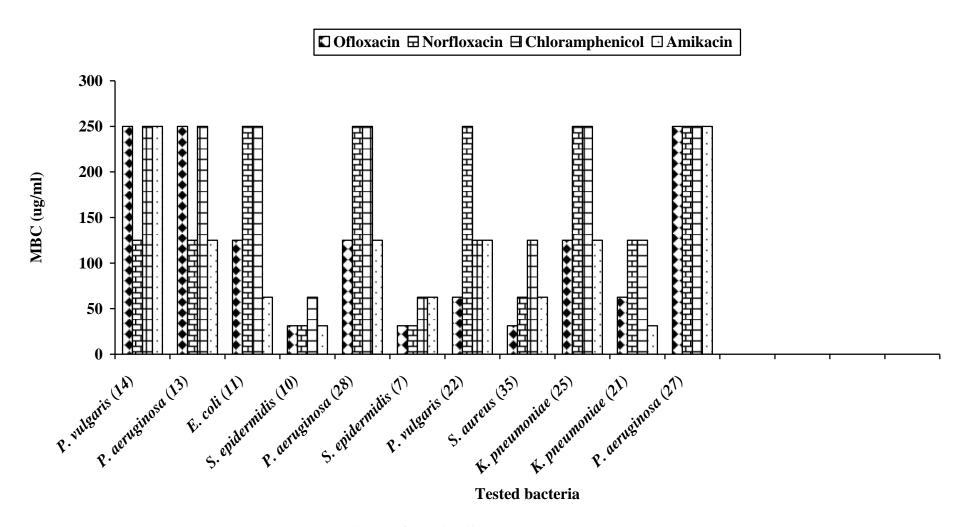


Fig. (5): Minimum bactericidal concentration (MBCs) (µg/ml) of different antibiotics against tested bacteria.

# 9- Antagonistic effect of different plant extracts (cold & boiling water and alcohol extract) against selected bacterial isolates.

In this experiment, an attempt was made to test the antagonistic effect of different plant extracts (rosemary, orange peel, garlic, lemon grass, peppermint, spearmint, marjoram, thyme, clove, fennel, ginger, cinnamon, henna and chamomile), as cold water extract, boiling water extract and alcoholic extract against selected clinical bacterial isolates *E. coli* number 11, *P. aeruginosa* number 13, 27 & 28, *K. pneumoniae* number 21 & 25, *P. vulgaris* number 14 & 22 and *S. epidermidis* number 10 & 7, and *S. aureus* number 35, as illustrated in table (9, 10 & 11) and photos No. (4, 5 & 6).

## 9. a- Antagonistic effect of alcoholic plant extracts

The results given in table (9) showed that the alcoholic extract of ginger has not any antagonistic effect against tested clinical bacterial isolates. On the other hand chamomile, cinnamon, and spearmint extracts showed weak antagonistic effect on most bacterial isolates.

In generally thyme, peppermint, lemon grass and henna showed moderate effect against growth of most clinical bacterial isolates which give less than 20 mm inhibition zone.

Rosemary, clove, orange peel, marjoram, fennel and garlic alcoholic extract were given strong antagonistic effect against growth of most tested bacterial isolates, where in garlic extract give 25, 25, 20, 20, 20, 28 and 28 mm inhibition zones against *Proteus vulgaris* number 22, *S. epidermidis* number 7 & 10, *K. pneumoniae* number 25, *P. aeruginosa* number 27 & 13, and *E. coli* number 11, respectively. Fennel extract gave 20 mm against growth of *S. epidermidis* number 10. Marjoram extract gave 20, 20, and 20 mm against

growth of *S. epidermidis* number 10, *Proteus vulgaris* number 22 and *S. aureus* number 35. Orange peel extract gave 20 mm inhibition zones against *S. epidermidis* number 10. Clove extract gave 20, 20 and 20 mm inhibition zone against *Proteus vulgaris* number 14 and *K. pneumoniae* number 21 & 25. Rosemary extract gave 20, 20, 20 and 20 mm inhibition zones against *E. coli* number 11, *P. aeruginosa* number 13 and *K. pneumoniae* number 21 & 25, respectively.

#### 9. b- Antagonistic effect of boiling water plant extracts

The results given in table (10) showed that the boiling water extracts of garlic and spearmint have not any antagonistic effect against tested clinical bacterial isolates. On the other hand fennel, orange peel and ginger extracts showed weak antagonistic effect on most bacterial isolates.

In generally chamomile, marjoram, lemon grass, henna, peppermint and cinnamon showed moderate effect against growth of most clinical bacterial isolates which give less than 20 mm inhibition zone.

Rosemary, thyme and clove boiling water extract were given strong antagonistic effect against growth of most tested bacterial isolates, where in rosmary extract give 20 mm inhibition zones against *E. coli* number 11. Thyme extract gave 20 mm against growth of *P. aeruginosa* number 13. Clove extract gave 20, 20, 21 and 21 mm against growth of *K. pneumoniae* number 21, *Proteus vulgaris* number 22, *S. aureus* number 35, *S. epidermidis* number 10 and *E. coli* number 11, respectively.

### 9. c- Antagonistic effect of cold water extracts

The results given in table (11) showed that the cold water extracts of spearmint, have not any antagonistic effect against tested clinical bacterial

isolates. On the other hand marjoram, ginger, cinnamon, orang peel, chamomile and fennel extracts showed weak antagonistic effect on most bacterial isolates, but cold water extract of cinnamon give strong antagonistic activity against *P. aeruginosa* number 27 (20 mm).

In generally henna, thyme, peppermint, lemon grass and rosemary showed moderate effect against growth of most clinical bacterial isolates which give less than 20 mm inhibition zone.

Garlic and clove cold water extract were given strong antagonistic effect against growth of most tested bacterial isolates, where in garlic extract give 30, 20, 25, 25 and 28 mm inhibition zones against *S. epidermidis* number 7 & 10, *E. coli* number 11, *P. vulgaris* number 22 and *K. pneumoniae* number 25, respectively. Clove extract gave 22 mm against growth of *K. pneumoniae* number 25 and 20 mm against *K. pneumoniae* number 21 and *P. vulgaris* number 22, respectively.

Table (9): Diameter of inhibition zone (mm) of different plant extracts (extraction by methanol) against clinical bacterial isolates.

			Diameter of inhibition zones (mm)												
Bacterial isolates	No.	Rosemary	Orange peel	Garlic	Lemon grass	Peppermint	Spearmint	Marjoram	Thyme	Henna	Cinnamon	Clove	Fennel	Ginger	Chamomile
S. epidermidis	7	18	ND	25	ND	18	ND	11	17	10	11	17	10	ND	11
S. epidermidis	10	19	20	20	17	18	ND	20	14	12	ND	16	20	ND	13
E. coli	11	20	ND	28	ND	17	15	11	15	16	12	17	11	ND	15
P. aeruginosa	13	20	10	ND	13	18	ND	15	18	16	12	20	11	ND	10
P. vulgaris	14	19	11	10	11	18	ND	16	16	15	12	20	10	ND	10
K. pneumoniae	21	20	11	28	12	18	ND	12	10	13	ND	18	12	ND	ND
P. vulgaris	22	11	10	25	12	ND	ND	20	11	11	ND	17	11	ND	ND
K. pneumoniae	25	20	ND	20	10	16	ND	10	16	10	ND	20	13	ND	ND
P. aeruginosa	27	10	11	20	10	15	ND	18	10	15	ND	10	13	ND	ND
P. aeruginosa	28	15	ND	ND	14	18	ND	10	16	ND	10	16	12	ND	17
S. aureus	35	18	10	ND	16	17	ND	20	10	ND	ND	17	ND	ND	10

ND = Not detect

Table (10): Diameter of inhibition zone (mm) of different plant extracts (extraction by boiling water) against clinical bacterial isolates.

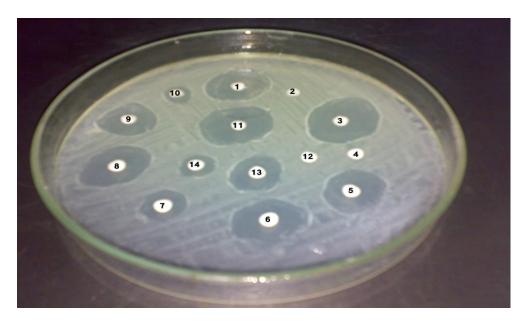
			Diameter of inhibition zones (mm)												
Bacterial isolates	No.	Rosemary	Orange peel	Garlic	Lemon grass	Peppermint	Spearmint	Marjoram	Thyme	Henna	Cinnamon	Clove	Fennel	Ginger	Chamomile
S. epidermidis	7	10	ND	ND	11	15	ND	11	12	12	12	17	ND	10	11
S. epidermidis	10	17	10	10	15	16	ND	16	15	14	13	21	ND	15	11
E. coli	11	20	11	ND	14	17	ND	ND	11	15	13	21	10	10	13
P. aeruginosa	13	15	12	ND	13	18	ND	15	20	18	13	15	10	12	16
P. vulgaris	14	13	11	ND	12	18	ND	15	19	16	11	17	10	10	16
K. pneumoniae	21	17	12	ND	12	15	ND	16	14	15	13	20	10	ND	12
P. vulgaris	22	11	11	10	10	10	ND	ND	ND	14	15	20	ND	ND	ND
K. pneumoniae	25	15	ND	ND	ND	17	ND	12	ND	ND	10	15	ND	ND	ND
P. aeruginosa	27	16	ND	ND	13	12	12	16	10	11	12	16	ND	ND	12
P. aeruginosa	28	16	ND	ND	12	12	ND	12	ND	ND	11	19	11	ND	ND
S. aureus	35	18	ND	ND	10	14	ND	15	13	ND	12	20	10	ND	12

ND = Not detect

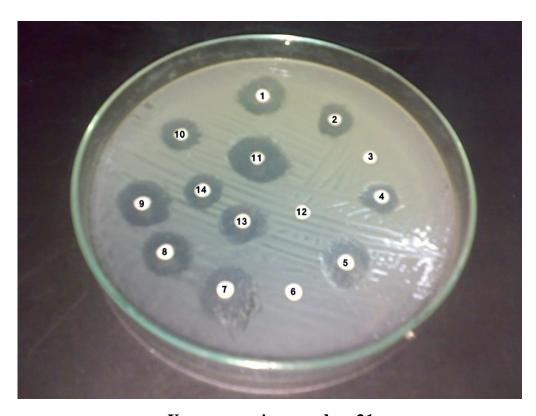
Table (11): Diameter of inhibition zone (mm) of different plant extracts (extraction by cold water) ) against clinical bacterial isolates.

			Diameter of inhibition zones (mm)												
Bacterial isolates	No.	Rosemary	Orange peel	Garlic	Lemon grass	Peppermint	Spearmint	Marjoram	Thyme	Henna	Cinnamon	Clove	Fennel	Ginger	Chamomile
S. epidermidis	7	11	ND	30	ND	ND	ND	10	ND	ND	ND	15	ND	ND	12
S. epidermidis	10	16	ND	20	12	12	ND	ND	10	16	10	15	12	ND	12
E. coli	11	14	ND	25	ND	11	ND	ND	ND	15	10	18	ND	ND	11
P. aeruginosa	13	16	ND	15	10	12	ND	ND	10	15	ND	17	10	15	15
P. vulgaris	14	14	ND	18	10	14	ND	ND	10	13	ND	17	11	13	14
K. pneumoniae	21	14	ND	15	ND	12	ND	ND	12	11	ND	20	10	12	ND
P. vulgaris	22	ND	10	25	10	ND	ND	15	12	ND	15	20	10	ND	ND
K. pneumoniae	25	ND	ND	28	12	ND	ND	ND	10	15	13	22	ND	ND	ND
P. aeruginosa	27	11	ND	15	11	15	ND	15	11	ND	20	17	ND	ND	ND
P. aeruginosa	28	11	ND	15	13	11	ND	10	ND	ND	ND	16	ND	ND	ND
S. aureus	35	15	ND	ND	ND	12	ND	ND	12	ND	ND	ND	ND	ND	12

ND = Not detect



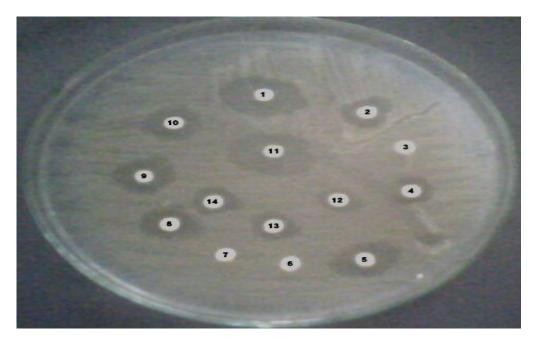
E. coli number 11



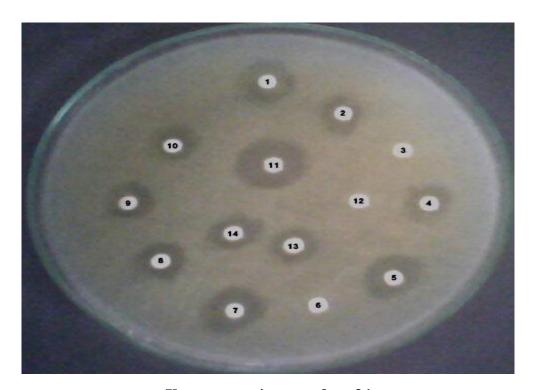
K. pneumoniae number 21

Photos No. (4): Effect of alcoholic plant extracts against bacterial isolates by disc diffusion method.

1- Rosemary, 2- Orange peel, 3- Garlic, 4- Lemon grass, 5- Peppermint, 6- Spearmint, 7- Marjoram, 8- Thyme, 9- Henna, 10- Cinnamon, 11- Clove, 12- Ginger, 13- Chamomile, 14- Fennel.



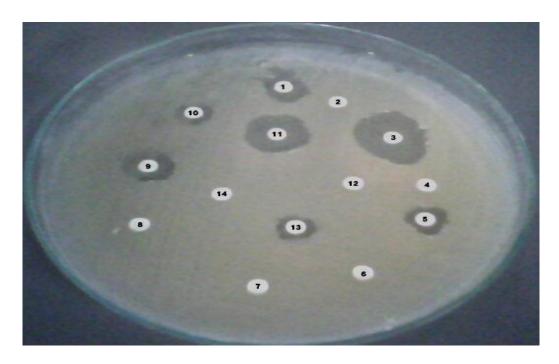
E. coli number 11



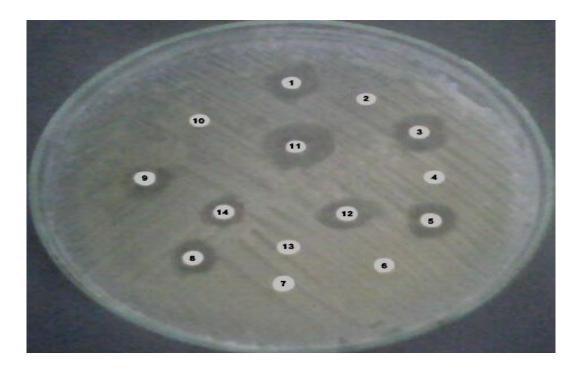
K. pneumoniae number 21

Photos No. (5): Effect of boiling water plant extracts against bacterial isolates by disc diffusion method.

1- Rosemary, 2- Orange peel, 3- Garlic, 4- Lemon grass, 5- Peppermint, 6- Spearmint, 7- Marjoram, 8- Thyme, 9- Henna, 10- Cinnamon, 11- Clove, 12- Ginger, 13- Chamomile, 14- Fennel.



E. coli number 11



K. pneumoniae number 21

Photos No. (6): Effect of cold water plant extracts against bacterial isolates by disc diffusion method.

1- Rosemary, 2- Orange peel, 3- Garlic, 4- Lemon grass, 5- Peppermint, 6- Spearmint, 7- Marjoram, 8- Thyme, 9- Henna, 10- Cinnamon, 11- Clove, 12- Ginger, 13- Chamomile & 14- Fennel.

### 10- The effect of combination between different plant extracts and MICs of some selected antibiotics on clinical bacterial isolates

The plant extracts that used in this experiment were rosemary, garlic, peppermint and clove which combined with the MICs of ofloxacin and amikacin for each clinical bacterial isolates to study the effect of combination between them and their action against bacterial isolates will increase (synergistic effect) than effect of singly used or not affected.

Extraction of rosemary, peppermint and garlic by alcohol, while extraction of clove by boiling water, were used which recorded the best extraction gave the highest antagonistic activities against clinical bacterial isolates.

The result of combination effect were recorded in table (12) and photos No. (7), which indicated that the combination between MIC of ofloxacin antibiotics with plant extract rosemary clearly synergistic action against *E. coli* number 11, *P. aeruginosa* number 28, *K. pneumoniae* number 21 & 25, *P. vulgaris* number 14 & 22 and *S. epidermidis* number 7 & 10 more than singly used of antibiotic ofloxacin or rosemary extract. On the other hand the antagonistic effect clearly illustrated with combination between rosemary extract and ofloxacin against *S. aureus* number 35, and *P. aeruginosa* number 13 & 27, which recorded inhibition action against these bacterial isolates less than singly used antibiotics.

Synergistic effect of combination between ofloxacin and clove, were recorded against *P. vulgaris* number 22, *P. aeruginosa* number 28 and *K. pneumoniae* number 25. On the other hand the antagonistic effect clearly illustrated with combination between clove extract and ofloxacin against *S. epidermidis* number 7 & 10, *E. coli* number 11, *P. aeruginosa* number 13 & 27,

S. aureus number 35, K. pneumoniae number 21 and P. vulgaris number 14. Synergistic effect of combination between ofloxacin and garlic did not record any inhibition zones.

The result of combination effect were recorded in table (13) and photos No. (8), which indicated that the combination between MIC of amikacin antibiotics with plant extract rosemary clearly synergistic action against *E. coli* number 11, *P. aeruginosa* number 28, *K. pneumoniae* number 25, *P. vulgaris* number 22, *S. epidermidis* number 10 and *S. aureus* number 35, more than singly used of antibiotic amikacin or rosemary extract. On the other hand the antagonistic effect clearly illustrated with combination between rosemary extract and amikacin against *K. pneumoniae* number 21, *P. aeruginosa* number 13 & 27, *S. epidermidis* number 7 and *P. vulgaris* number 14, which recorded antagonistic action against these bacterial isolates less than singly used antibiotics.

Synergistic effect of combination between amikacin and clove, were recorded against *S. epidermidis* number 7 & 10 which obtained, *P. vulgaris* number 14 & 22, *P. aeruginosa* number 13 & 27 and *K. pneumoniae* number 25 & 28. Antagonistic effect was clearly obtained at clove against *E. coli* number 11 and *P. aeruginosa* number 28.

Synergistic effect of combination between amikacin and garlic did not record any inhibition zones.

The statistical analysis for selected plant extracts combined with antibiotics, incase of ofloxacin, peppermint is significant but rosemary and clove are non significant and incase of amikacin clove is highly significant while garlic is significant but rosemary and peppermint are non significant.

Table (12): Diameter of inhibition zone (mm) of combination between different plant extracts and MICs of ofloxacin.

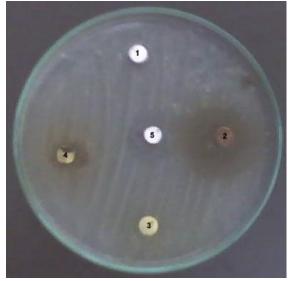
				Diameter of inhibition zone (mm)					
Bacterial isolates	Ofloxac	in MICs	Rosemary	Garlic	Clove	Peppermint			
	No	μg/ml	IZ						
P. vulgaris	14	250	12	ND	ND	20	10		
P. aeruginosa	13	125	10	17	ND	20	11		
E. coli	11	125	13	ND	ND	15	ND		
S. epidermidis	10	7.8125	18	15	ND	19	14		
P. aeruginosa	28	62.5	16	16	ND	12	10		
S. epidermidis	7	31.25	18	16	ND	17	ND		
P. vulgaris	22	62.5	15	11	ND	14	12		
S. aureus	35	7.8125	15	15	ND	19	13		
K. pneumoniae	25	125	13	15	ND	17	11		
K. pneumoniae	21	31.25	15	11	ND	13	12		
P. aeruginosa	27	125	11	11	ND	14	14		
$\overline{X}$ ±SD			14.2 <u>+</u> 2.6	14.1 <u>+</u> 2.4		16.3 <u>+</u> 2.9	11.9 ±1.5		
t			0.06		1.84	2.3			
P		0.95 NS		0.07 NS	0.03 S				

Table (13): Diameter of inhibition zone (mm) of combination between different plant extracts and MICs of amikacin.

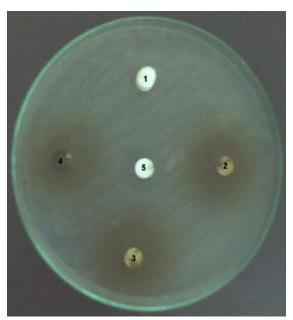
				Dia	meter	of inhibi (mm)	tion zone
Bacterial isolate	Amikacin MICs		Rosemary	Garlic	Clove	Peppermint	
	No	μg/ml	IZ				
P. vulgaris	14	125	15	16	15	20	17
P. aeruginosa	13	62.5	12	15	12	17	14
E. coli	11	62.5	13	10	10	15	13
S. epidermidis	10	31.25	13	13	ND	15	12
P. aeruginosa	28	125	15	16	ND	18	16
S. epidermidis	7	31.25	13	11	14	13	10
P. vulgaris	22	62.5	12	12	10	14	11
S. aureus	35	62.5	14	14	ND	15	12
K. pneumoniae	25	62.5	13	15	10	18	17
K. pneumoniae	21	31.25	10	12	10	15	15
P. aeruginosa	27	125	15	13	10	15	16
$\overline{X}$ ±SD			13.2 <u>+</u> 1.5	13.3 <u>+</u> 2	11.3 <u>+</u> 2	15.9 +2.1	13.9 +2.5
t		_	0.23	2.19	3.5	0.82	
P		0.8 NS	0.04 S	0.002 HS	0.57 NS		



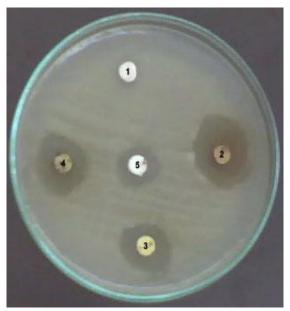
S. epidermidis number 10



E. coli number 11



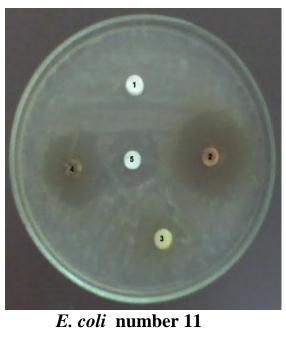
P. aeruginosa number 13

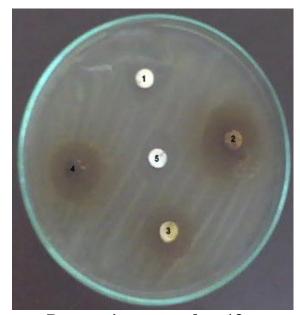


K. pneumoniae number 21

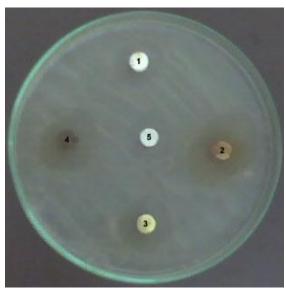
Photos No. (7): Combination between plant extracts and ofloxacin antibiotic against bacterial isolates by disc diffusion method.

1- Garlic, 2- Clove, 3- Rosemary, 4- Peppermint, 5- MIC of ofloxacin.





P. aeruginosa number 13



P. vulgaris number 14



K. pneumoniae number 21

Photos No. (8): Combination between plant extracts and amikacin antibiotic against bacterial isolates by disc diffusion method.

1- Garlic, 2- Clove, 3- Rosemary, 4- Peppermint, 5- MIC of amikacin.

#### 11- The effect of different volatile oils against different bacterial isolates

In this experiment different volatile oils were used as peppermint, spearmint, lemon, thyme, clove, rosemary, garlic, marjoram, fennel and eucalyptus against selected clinical bacterial isolates *E. coli* number 11, *P .aeruginosa* number 13, 27 & 28, *K. pneumoniae* number 21, 25, *P. vulgaris* number 14 & 22, *S. epidermidis* number 10 & 7 and *S. aureus* number 35.

The result given in table (13), fig. (6) and photos No. (9), showed that the fennel, eucalyptus, lemon grass, spearmint and clove showed weak antagonistic effect on most of bacterial isolates but clove of volatile oil gives strong antagonistic activity against *P. aeruginosa* number 27 (26) mm.

In generally rosemary showed moderate antagonistic activity. Peppermint, thym, garlic and marjoram given strong antagonistic activity against growth of most tested bacterial isolates, where in peppermint give 50, 35, 30, 25, 25, 22 and 20 mm inhibition zones against *S. aureus* number 35, *K. pneumoniae* number 21 *P. aeruginosa* number 13 & 28, *K. pneumoniae* number 25 and *P. vulgaris* number 14, respectively. Where in thyme give 25 & 27 mm inhibition zones against *P. aeruginosa* number 28 and *S. aureus* number 35. Garlic give 30, 35, 30, 25 and 20 mm inhibition zones against *P. aeruginosa* number 13, *K. pneumoniae* number 21, *S. aureus* number 35, *P. aeruginosa* number 28 and *P. vulgaris* number 22, respectively. Marjoram gives 25 (mm) for *S. aureus* number 35 & *K. pneumoniae* number 21.

Table (14): Diameter of inhibition zone (mm) of different essential oils against pathogenic bacterial isolates

			Diameter of inhibition zones (mm)								
Bacterial isolates	No.	Peppermint	Spearmint	Lemon	Thyme	Clove	Rosemary	Garlic	Marjoram	Eucalyptus	Fennel
P. aeruginosa	27	22	15	12	10	26	ND	16	14	11	14
P. aeruginosa	28	25	10	13	25	10	12	25	15	11	11
S. aureus	35	50	10	11	27	10	20	35	25	ND	10
P. aeruginosa	13	30	14	15	15	10	14	30	16	16	11
K. pneumoniae	25	25	11	11	14	ND	ND	15	14	13	ND
K. pneumoniae	21	35	ND	ND	15	ND	12	30	25	ND	ND
P. vulgaris	14	20	ND	ND	14	ND	10	14	13	ND	ND
P. vulgaris	22	15	ND	ND	12	ND	ND	20	ND	ND	ND
S. epidermidis	10	17	ND	ND	18	ND	ND	11	ND	ND	ND
S. epidermidis	7	19	ND	ND	15	ND	10	12	ND	ND	ND
E. coli	11	ND	ND	ND	16	ND	ND	19	15	ND	ND

ND = Not detect

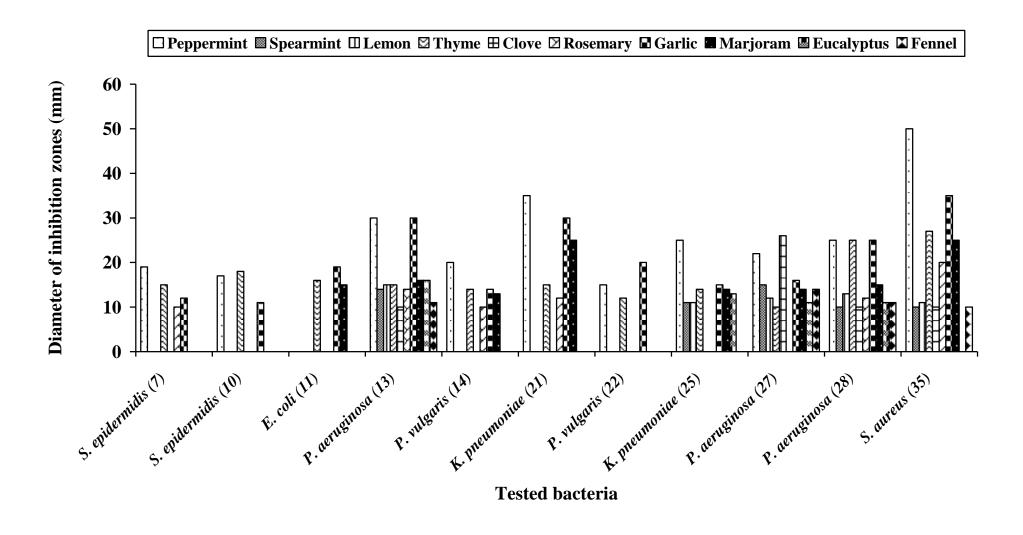
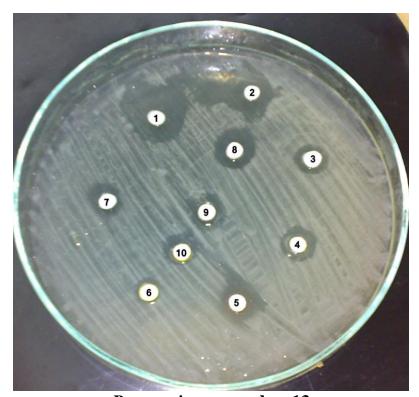


Fig. (6): Diameter of inhibition zones of different essential oils against tested bacterial isolates



S. epidermidis number 10



P. aeruginosa number 13

Photos No. (9): Effect of essential oils against bacterial isolates by disc diffusion method.

- 1- Peppermint, 2- Spearmint, 3- Lemon, 4- Thyme, 5- Clove, 6- Rosemary, 7- Garlic,
- 8- Marjoram, 9- Eucalyptus, 10- Fennel.

### 12- The effect of disinfectant on tested bacterial isolates.

In this experiment different disinfectants substances as betadine paint, mercrychrome, iodine, cetavlon, gentian paint and gawy stain, where tested for their antimicrobial activity against bacterial isolates by disc diffusion method as show in table (14), fig. (7), and photos No. (10).

Iodine and betadin showed the highest antibacterial activity against growth of most tested bacterial isolates, where iodine give 25, 24, 25, 23, 22, 26, 40, 33, 50, 33 and 40 mm inhibition zones against *S. epidermidis* 7 & 10, *E. coli* 11, *P. aeruginosa* 13, *P. vulgaris* 14, *K. pneumoniae* 21, *P. vulgaris* 22, *K. pneumoniae* 25, *P. aeruginosa* 27 & 28 and *S. aureus* 35, respectively. Where in betadin give 38, 20, 25, 20, 20, 15, 35, 30, 33, 30 and 35 mm inhibition zones against *S. epidermidis* 7 & 10, *E. coli* 11, *P. aeruginosa* 13, *P. vulgaris* 14, *K. pneumoniae* 21, *P. vulgaris* 22, *K. pneumoniae* 25, *P. aeruginosa* 27 & 28 and *S. aureus* 35, respectively. Gentian paint, mercrychrome and gawy stain showed moderate antibacterial activity against growth of most tested bacterial isolates but cetavlon hasn't any antibacterial activity against growth of most tested bacterial isolates.

Table (15): Diameter of inhibition zones (mm) of different disinfectants against pathogenic bacterial isolates.

		Diameter of inhibition zones (mm)						
Bacterial isolates	No	Betadine	Gawy	Gentian	Cetavlon	Mercrychrome	Iodine	
P. aeruginosa	13	20	15	12	ND	20	23	
K. pneumoniae	21	15	15	15	ND	15	26	
P. aeruginosa	27	33	30	23	ND	20	50	
K. pneumoniae	25	30	25	20	ND	20	33	
P. vulgaris	14	20	20	16	ND	10	22	
E. coli	11	25	17	17	ND	18	25	
P. aeruginosa	28	30	23	15	ND	18	33	
S. epidermidis	10	20	25	15	ND	15	24	
S. aureus	35	35	21	18	ND	25	40	
P. vulgaris	22	35	25	18	ND	22	40	
S. epidermidis	7	38	20	15	ND	17	25	

ND = Not detect

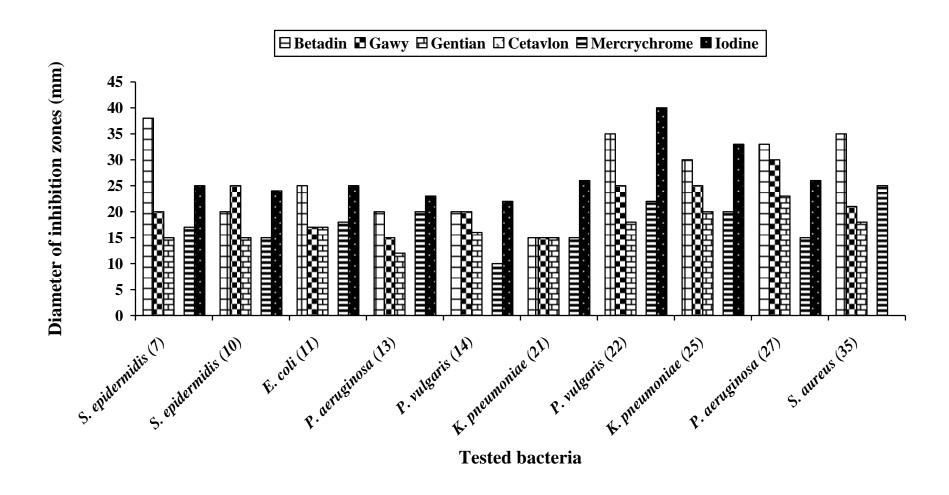
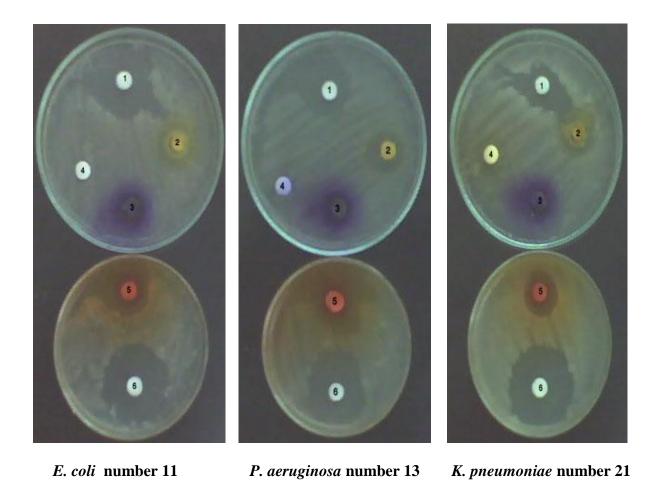


Fig. (7): Diameter of inhibition zones of different disinfectants against tested bacterial isolates.



**Photo No. (10): The effect of disinfectants on tested bacterial isolates** 1- Betadin, 2- Gawy, 3- Gentian, 4- Cetavlon, 5- Mercrychrome, 6- Iodine.

# 13- Antibacterial activities of honey bees against bacterial isolates from bed sores patients.

The different types of honey bees were used to study their effect on growth of bacterial isolates. The data obtained in table (15), fig. (8), and photos No. (11), illustrated that, date honey showed the highest antibacterial activity, where it gives 21 mm inhibition zone against *E. coli* number 11, followed by seder honey, where it gives 19 mm inhibition zone against *S. epidermidis* number 10, the pond grain honey gives 18 mm inhibition zone against *E. coli* number 11. Citrus honey gives 17 mm inhibition zone against *E. coli* number 11, clover honey gives 16 mm inhibition zone against *P. aeruginosa* number 13 and albrdqoc honey gives 14 mm against *P. vulgaris* number 14 and *K. pneumoniae* number 25. On other side, *P. aeruginosa* number 28 is the less sensitive organism and *E. coli* is the most sensitive organism to all honey bees used in this study.

Table (16): Diameter of inhibition zone (mm) of different types of honey bees against pathogenic bacterial isolates.

		Diameter of inhibition zones (mm)							
Bacterial isolates	No.	The pond grain honey	Date honey	Citrus honey	Albrdqoc honey	Clover honey	Seder honey		
S. epidermidis	7	15	15	13	12	10	10		
P. aeruginosa	13	13	12	12	13	16	14		
K. pneumoniae	25	12	13	13	14	12	13		
K. pneumoniae	21	15	15	12	10	12	15		
E. coli	11	18	21	17	15	13	16		
S. epidermidis	10	15	12	12	11	12	19		
P. vulgaris	22	12	13	11	10	15	14		
P. vulgaris	14	12	12	13	14	9	11		
S. aureus	35	15	11	12	12	9	15		
P. aeruginosa	27	9	10	11	12	11	13		
P. aeruginosa	28	10	9	12	10	9	9		

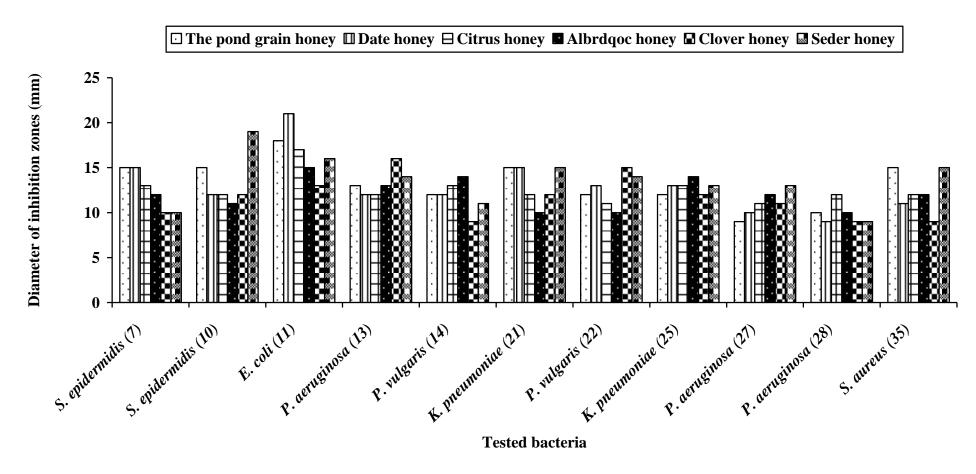


Fig. (8): Diameter of inhibition zones of different types of honey bees against tested bacterial isolates.



K. pneumoniae number 21



S. aureus number 35

Photos No. (11): Effect of different types of honey bees against bacterial isolates by disc diffusion method.

1- The pond grain honey, 2- Date honey, 3- Citrus honey, 4- Albrdqoc honey, 5- Clover honey, 6- Seder honey

# 14- Antibacterial activities of alcoholic extracts of propolise against bacterial isolates from bed sores patient.

The following concentration of alcoholic propolise extracts was prepared to prove the antibacterial activities as follow 100, 75, 50, and 25%. The obtained result illustrated by table (16), fig. (9), and photo No. (12), showed paper disc assay of different concentration of alcoholic extracts of propolise against bacterial isolates, where the highest concentration (100%) give the highest inhibition zones with all tested bacteria, followed by the concentration (75%) which give inhibition zones less than the highest concentration (100%), while incase the concentration (50%) it is moderate, affected only on some tested bacteria, but incase (25%) it hasn't any effect. From that the antibacterial activities of alcoholic extracts of propolise increased with increasing the concentration.

Table (17): Diameter of inhibition zone (mm) of different concentration of alcoholic extracts of propolise against pathogenic bacterial isolates.

Bacterial isolates		Diameter of inhibition zones (mm)									
	No.		Propolise concentration (%)								
		100	75	50	25						
K. pneumoniae	25	13	10	ND	ND						
S. epidermidis	10	11	8	ND	ND						
P. vulgaris	22	13	11	10	ND						
K. pneumoniae	21	20	13	8	ND						
P. aeruginosa	13	11	10	8	ND						
S. aureus	35	12	10	9	ND						
S. epidermidis	7	12	11	10	ND						
P. vulgaris	14	12	10	ND	ND						
E. coli	11	12	11	ND	ND						
P. aeruginosa	27	13	11	10	ND						

ND = Not detect

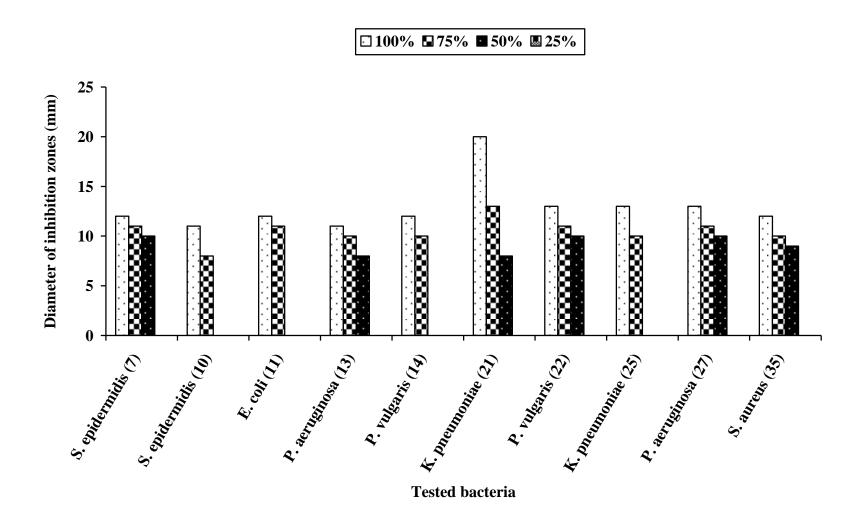
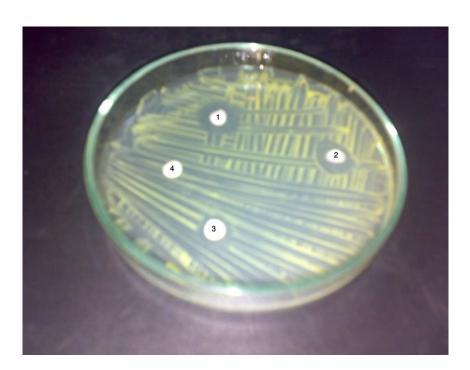


Fig. (9): Diameter of inhibition zone (mm) of different concentration of alcoholic extracts of propolise against tested bacterial isoltes



K. pneumoniae number 21



S. aureus number 35

Photos No. (12): Effect of different concentration of propolise against bacterial isolates by disc diffusion method.

1- 100 %, 2- 75 %, 3- 50 % & 4- 25 %